

IN THE SPECIFICATION

Please replace the paragraphs beginning on page 13, line 12, with the following amended paragraphs:

Fig. 2 is a figure showing a design of the oligonucleotide PRO-1F (SEQ ID NO:9) based on nucleotides 628 to 669 of SEQ ID NO:7 which encode residues 169 to 182 of SEQ ID NO:8.

Fig. 3 is a figure showing a design of the oligonucleotide PRO-2F (SEQ ID NO:10) based on nucleotides 1210 to 1251 of SEQ ID NO:7 which encode residues 363 to 376 of SEQ ID NO:8 and PRO-2R (SEQ ID NO:11).

Fig. 4 is a figure showing a design of the oligonucleotide PRO-4R (SEQ ID NO:12) based on nucleotides 1882 to 1923 of SEQ ID NO:7 which encode residues 587 to 600 of SEQ ID NO:8.

Please replace the paragraphs beginning on page 13, line 25, to the top of page 14, with the following amended paragraphs:

Fig. 11 is a figure comparing the amino acid sequences of the various proteases of PFUL (SEQ ID NO:8), TCES (SEQ ID NO:1) and Subtilisin (SEQ ID NO:45).

Please replace the paragraphs beginning on page 50, line 15, with the following amended paragraphs:

~~In~~ In addition, the enzymes obtained in the present invention hydrolyze succinyl-L-leucyl-L-leucyl-L-valyl-L-tyrosine-4-methylcoumarin-7-amide (Suc-Leu-Leu-Val-Tyr-MCA; SEQ ID NO:43) to produce a fluorescent material (7-amino-4-methylcoumarin).

~~Further,~~ Further, the enzymes obtained in the present invention hydrolyze succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine-p-nitroanilide (Suc-Ala-Ala-Pro-Phe-p-NA; SEQ ID NO:44) to produce a yellow material (p-nitroaniline).--

Please replace the paragraphs beginning on page 51, line 3, to page 55, line 16, with the following amended paragraphs:

~~The~~ The enzyme activity of the enzyme preparation TC-3 obtained in the present invention can be measured using as a substrate Suc-Leu-Leu-Val-Tyr-MCA (SEQ ID NO:43) (manufactured by Peptide Laboratory). That is, the enzyme preparation to be detected for the enzyme activity is appropriately diluted, to 20 μ l of the solution is added 80 μ l of a 0.1M sodium phosphate buffer (pH 7.0) containing 62.5 μ M Suc-Leu-Leu-Val-Tyr-MCA (SEQ ID NO:43), followed by incubating at 75 °C for 30 minutes. After the reaction is stopped by the addition of 20 μ l of 30% acetic acid, the fluorescent intensity is measured at the excitation wavelength

of 355 nm and the fluorescence wavelength of 460 nm to quantitate an amount of the generated 7-amino-4-methylcoumarin, and the resulting value is compared with that obtained when incubating without the addition of the enzyme preparation, to investigate the enzyme activity. The enzyme preparation TC-3 obtained by the present invention had the Suc-Leu-Leu-Val-Tyr-MCA (SEQ ID NO:43) hydrolyzing activity measured at pH 7.0 and 75 °C.

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In addition, the enzyme activity of the enzyme preparations NP-1, PT-1, NAPS-1 and NAPS-1S can be photometrically measured using Suc-Ala-Ala-Pro-Phe-p-NA (SEQ ID NO:44) (manufactured by Sigma) as a substrate. That is, an enzyme preparation to be detected for the enzyme activity was appropriately diluted, to 50 µl of the solution was added 50 µl of a 0.1M potassium phosphate buffer (pH 7.0) containing Suc-Ala-Ala-Pro-Phe-p-NA (SEQ ID NO:44) (Suc-Ala-Ala-Pro-Phe-p-NA-(SEQ ID NO:44) solution), followed by incubating at 95 °C for 30 minutes.

After the reaction was stopped by ice-cooling, the absorbance at 405 nm was measured to quantitate an amount of the generated p-nitroaniline, and the resulting value was compared with that when incubating without the addition of the enzyme preparation, to investigate the enzyme activity. Upon this, a 0.2 mM solution of Suc-Ala-Ala-Pro-Phe-p-NA (SEQ ID NO:44) was used for the enzyme preparations NP-1 and PT-1 and a 1 mM solution was used for the enzyme preparations NAPS-1 and NAPS-1S. The enzyme preparations NP-1, PT-1, NAPS-1 and NAPS-1S obtained by the present invention

have the Suc-Ala-Ala-Pro-Phe-p-NA (SEQ ID NO:44) hydrolyzing activity at measured pH 7.0 and 95 °C.

(3) Detection of activity on various substrates

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The activity of the enzyme preparations obtained in the present invention on the synthetic peptide substrates is confirmed by a method for measuring the enzyme activity described in the above (2). That is, the enzyme preparation TC-3 obtained in the present invention has the Suc-Leu-Leu-Val-Tyr-MCA (SEQ ID NO:43) hydrlyzing activity, and the enzyme preparations NP-1, PT-1, NAPS-1 and NAPS-1A have the Suc-Ala-Ala-Pro-Phe-p-NA (SEQ ID NO:44) hydrlyzing activity, respectively. In addition, the enzyme preparations NP-1, PT-1, NAPS-1 and NAPS-1S were investigated for the Suc-Leu-Leu-Val-Tyr-MCA (SEQ ID NO:43) hydrlyzing activity by the enzyme activity measuring method described in the above (2) used for the enzyme preparation TC-3, and it was shown that these enzyme preparations had the activity to degrade the substrates. Further, the enzyme preparation TC-3 was investigated for the Suc-Ala-Ala-Pro-Phe-p-NA (SEQ ID NO:44) hydrlyzing activity by the enzyme activity measuring method described in the above (2) used for the enzyme preparations NP-1 and PT-1, and the activity to degrade the substrate was recognized. In addition, the activity of the enzyme preparations obtained in the present invention on gelatin can be detected by confirming the degradation of gelatin by an enzyme on the SDS-polyacrylamide gel. That is, the enzyme preparation to be

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B detected for the enzyme activity was appropriately diluted, to 10 µl of the sample solution was added 2.5 µl of a sample buffer (50 mM Tris-HCl, pH 7.5, 5% SDS, 5% 2-mercaptoethanol, 0.005% Bromophenol Blue. 50% glycerol), followed by treatment at 100 °C for 5 minutes and electrophoresis using 0.1% SDS-10% polyacrylamide gel containing 0.05% gelatin. After the completion of run, the gel was soaked in a 50 mM potassium phosphate buffer (pH 7.0), and incubated at 95 °C for 3 hours to carry out the enzyme reaction. Then, the gel was stained in 2.5% Coomassie Brilliant Blue R-250, 25% ethanol and 10% acetic acid for 30 minutes, and transferred in 7% acetic acid to remove the excess dye over 3 to 15 hours. The presence of the protease activity was detected by the fact that gelatin is hydrolyzed by a protease into peptides which are diffused out of the gel and, consequently, the relevant portion of the gel was not stained with Coomassie Brilliant Blue. The enzyme preparations TC-3, NP-1, PT-1, NAPS-1 and NAPS-1S obtained by the present invention had the gelatin hydrolyzing activity at 95 °C. --

Please replace the paragraphs beginning on page 56, line 19, to page 58, line 15 with the following amended paragraphs:

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B --The optimum pH of the enzyme preparation TC-3 obtained by the present invention was investigated by the enzyme activity measuring method shown in the above (2). That is, the Suc-Leu-Leu-Val-Tyr-MCA (SEQ ID NO:44) solutions were prepared using the

buffers having various pHs, and the enzyme activities obtained by using these solutions were compared. As a buffer, a sodium acetate buffer was used at pH 3 to 6, a sodium phosphate buffer at pH 6 to 8, a sodium borate buffer at pH 8 to 9, and a sodium phosphate-sodium hydroxide buffer at pH 10 to 11. As shown in Fig. 20, the enzyme preparation TC-3 shows the activity at pH 5.5 to 9, and the optimum pH was pH 7 to 8. That is, Fig. 20 is a figure showing the relationship between the activity of the enzyme preparation TC-3 obtained in the present invention and pH, and the ordinate shows the relative activity (%) and the abscissa shows pH.

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In addition, the optimum pH of the enzyme preparation NP-1 obtained in the present invention was investigated by the enzyme activity measuring method shown in the above (2). That is, the Suc-Ala-Ala-Pro-Phe-pNA (SEQ ID NO:44) solutions were prepared by using the buffers having various pHs, and the enzyme activities obtained by using these solution were compared. As a buffer, a sodium acetate buffer was used at pH 4 to 6, a potassium phosphate at pH 6 to 8, a sodium borate buffer at pH 5 to 10, and a sodium phosphate-sodium hydroxide buffer at pH 10.5. As shown in Fig. 21, the enzyme preparation NP-1 shows the activity at pH 5 to 10, and the optimum pH was pH 5.5 to 8. That is, Fig. 21 is a figure showing the relationship between the activity of the enzyme preparation NP-1 obtained in the present invention and pH, and the

ordinate shows the relative activity (%) and the abscissa shows pH.

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Further, the optimum pH of the enzyme preparation NAPS-1 obtained in the present invention was investigated by the enzyme activity measuring method shown in the above (2). That is, the Suc-Ala-Ala-Pro-Phe-pNA (SEQ ID NO:44) solutions were prepared by using the buffers having various pHs, and the enzyme activities obtained by using these solution were compared. As a buffer, a sodium acetate buffer was used at pH 4 to 6, a potassium phosphate at pH 6 to 8, a sodium borate buffer at pH 8.5 to 10. As shown in Fig. 22, the enzyme preparation NAPS-1 shows the activity at pH 5 to 10, and the optimum pH was pH 6 to 8. That is, Fig. 22 is a figure showing the relationship between the activity of the enzyme preparation NAPS-1 obtained in the present invention and pH, and the ordinate shows the relative activity (%) and the abscissa shows pH.
